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COMPLETE SPECIFICATION

Vitamin emulsions

We, MERCK & CO. INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to aqueous vitamin emulsions and procedures for preparing them. More particularly, the invention relates to aqueous emulsions suitable for intravenous injection.

While it has heretofore been possible to administer the water-soluble vitamins by injection, practical procedures for injecting water-insoluble vitamins such as vitamins A, D, K and K_1, hereinafter referred to as the oily vitamins, have not been available.

In accordance with the invention, there is provided a stable oily vitamin emulsion for administration by intravenous injection which comprises an oily vitamin and lecithin dispersed in water, in which the dispersed particles are predominantly of a diameter in the range from 0.1 to 0.5 microns.

The invention also provides a process for producing such emulsions.

While lecithin can be obtained from various sources it has been found that soy bean lecithin and egg lecithin give the best results in preparing the stable vitamin emulsions. These lecithins are physiological substances which can be intravenously injected without adverse effect in the amounts contemplated in the vitamin emulsions of the invention and have the further advantage of being unaffected by autoclaving of the completed emulsion.

The emulsions of the invention may be prepared by admixing (1) a solution of the oily vitamin in an aliphatic alcohol whose molecule contains up to four carbon atoms, (2) a solution of lecithin in a volatile organic solvent, and (3) water, emulsifying the resulting mixture by agitation, and removing the organic solvents from the resulting emulsion.

In preparing the solution of the oily vitamin in the alcohol, alcohols containing up to four carbon atoms in the molecule are used. It is preferable to use propanol or isopropanol, since these alcohols provide an optimum combination of solubility for oily vitamins and ease of evaporation from the completed emulsion. Butanols can also be used with advantage, but higher alcohols than butanol cannot be so readily removed by evaporation from the completed emulsion. Methanol and ethanol, although also usable, are less preferred because, being poorer solvents for the oily vitamins, they must be used in substantially larger amounts.

The oily vitamins can be employed singly and in compatible combination to provide stable emulsions having the properties or combination of properties desired in various preparations for intravenous injection.

The alcoholic solution of oily vitamin is then combined with a solution of lecithin in a volatile organic solvent which must also be capable of dissolving the vitamin to be emulsified. This solvent acts both to dissolve the lecithin and to increase the dispersibility of the oily vitamin in water during emulsification. It is preferred to use chloroform as the solvent for dissolving lecithin, but other, preferably non-alcoholic, solvents can be used for this purpose, including particularly ethyl ether and petroleum ether. In this connection, the volatility of the solvent should be such that the solvent is easily removed after formation of the emulsion by evaporation under vacuum.

According to a preferred procedure, the lecithin-vitamin-solvent mixture is then stirred with water of a volume equivalent to about one-and-one-half to two times the total volume of vitamin and solvent. The

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stirring is best effected by a high speed mechanical stirrer operating at 8000-9000 revolutions per minute, the stirring being continued for a period of time required to elevate the temperature of the mixture from about room temperature to about 43-45°C. The stirring speed and the temperature at which stirring is stopped appear to be primary factors in obtaining a stable emulsion. If stirring is stopped before reaching a temperature of 43-45°C., the particle size of the dispersed phase may be too large to provide a stable emulsion, whereas continuing the stirring until the temperature substantially exceeds 45°C. may result in a particle size too small for optimum stability.

When following the preferred procedure just described, the emulsion resulting from the stirring which is at a temperature of 43-45°C., is cooled to about 5°C. and then subjected to low pressure evaporation at a pressure of 1-5 mm. mercury and at temperatures ranging from an initial temperature of about 15-20°C. to a final temperature of about 45-50°C. The evaporation is advantageously carried out in two stages, the initial stage requiring about half the total evaporation time being conducted at a temperature of about 15-20°C. and effecting a removal of about 80% of the organic solvents. In the second stage, the temperature is gradually elevated to 45-50°C. and evaporation continued until emulsion is free of chloroform and other organic solvents. During the evaporation, a substantial quantity of water is also removed and the completed emulsion must generally be adjusted to the desired concentration by addition of water.

It has been found that addition of water at this stage or before the evaporation is started does not impair the stability of the final emulsion. In fact it is frequently advantageous, after initial emulsion formation by stirring until a temperature of 43-45°C. is reached, to add a quantity of water at a temperature of about 40-45°C. while stirring the emulsion in order to provide a substantial excess of water during the evaporation step. This addition of water does not alter the particle size of the dispersed phase but serves to prevent undue concentration of the emulsion during the evaporation step. In diluting the emulsion as above described, a quantity of water equivalent to the total volume of the emulsion can be employed.

The most stable emulsions are those in which the dispersed particles have a diameter within the range of about 0.1-0.5 microns. This particle size in the dispersed phase also provides an emulsion which may be injected intravenously without difficulty. An important factor in the control of particle size in the manner of mixing by high speed stirring as above described.

The size of the dispersed particles will also depend in part upon the relative volumes of oily vitamins, organic solvents, and water subjected to mechanical agitation. As previously pointed out, the volume of water used in forming the emulsions should preferably be about one-and-one half to two times the total volume of oily vitamin and organic solvents. By way of illustration, stable emulsions of oily vitamins can be prepared when the volume ratios of oily-vitamin : organic-solvents : water are within the range of 1 : 1 : 2 to about 1 : 5 : 12 with an optimum ratio of about 1 : 5 : 9. It is preferred not to use a total volume of organic solvents less than the amount of oily vitamin to be emulsified, as the mixture becomes unduly viscous and difficult to emulsify.

Increasing the proportion of organic solvents to oily vitamin produces a less viscous mixture which is more readily emulsified, but increasing the proportion beyond about five volumes of organic solvents to each volume of oily vitamin serves no useful purpase in the emulsification step, while at the same time introducing a problem of removing an excessive amount of organic solvents from the completed emulsion.

Of the total volume of organic solvents employed, preferably approximately one-third is chloroform or other solvent for the lecithin, while approximately two-thirds is isopropanol or other lower alkyl alcohol which is a solvent for the oily vitamin. It should be noted that these proportions can be varied, particularly as the relative amounts of lecithin and oily vitamin are varied. With smaller amounts of lecithin, the chloroform or other lecithin solvent may comprise as little as one-fourth of the total quantity of solvents, or as the amount of lecithin is increased, the quantity of chloroform may be as much as one-half the total quantity of organic solvents.

The preferred volume ratio of lecithin to oily vitamin is about one-two parts lecithin to five parts of the oily vitamin. Thus, in preparing vitamin emulsions of about 5% vitamin concentration, i.e., a concentration generally suitable for administration by injection, the lecithin will constitute only about 1 to 2% of the emulsion. The amount of lecithin introduced in normal dosages of 1-3 cc. of oily vitamin emulsions is insufficient to produce adverse side effects.

Since the emulsions are intended primarily for parenteral injection, it will be understood that appropriate steps should be taken to assure that the products are sterile and also that they are substantially free of pyrogens. In this connection, it should be noted that the emulsions are not adversely affected by extended heating at 120°C. and that

sterilisation can therefore be effected by autoclaving. The control is best carried out by employing pyrogen-free components in making the initial emulsion and carrying out successive steps under essentially pyrogen-free conditions.

The following examples will show how procedures for preparing the stable vitamin emulsions can be carried out but it will be understood that these examples are given by way of illustration and not of limitation.

Example 1

A 100 gram portion of soya bean lecithin was treated with 500 cc. of ethyl ether and centrifuged in order to remove denatured proteins and other insoluble material. Approximately one liter of acetone was slowly added to the ether solution and after 24 hours standing the solvents were decanted from the precipitated lecithin. The precipitate was further washed twice by decantation with one-liter portions of acetone, decantation following 24 hours standing in each case. The lecithin was filtered and dried in vacuo at room temperature. This purification procedure reduces the pyrogen content below 0.05 units per 60 milligrams.

Ten grams of purified lecithin containing not more than 0.05 pyrogens units per 60 milligrams of lecithin was dissolved in 90 cc. of chloroform in a 2-liter beaker. To this solution was first added 50 grams of vitamin K_1 dissolved in 170 cc. of isopropanol, and then 450 cc. of pyrogen-free distilled water at 25°C. The mixture was then stirred by means of a small mechanical stirrer at 8000-9000 revolutions per minute. The temperature rose gradually during stirring. When the temperature reached about 45°C., 730 cc. of water was added and stirring continued for approximately five minutes. Temperature of added water was 40-45°C.

Evaporation of the emulsion to remove the organic solvents was accomplished in a long-tube evaporator. The emulsion was cooled to 5°C. and evaporation in the long-tube evaporator allowed to proceed at a pressure of 1-5 mm. of mercury. The temperature of the circulating water in the evaporator at the beginning was 15-20°C. The initial stage of the evaporation process, during which approximately 80% of the solvents was removed, required approximately one-half hour. The temperature of the circulating water was then slowly raised to 45-50°C. and evaporation continued (approximately one-half hour) until the emulsion was found to be free of chloroform. During the evaporation, in addition to the removal of chloroform and isopropanol, approximately 200 cc. of water was removed. A measurement of the volume of emulsion after the concentration step indicates the amount of water to be added at this point, to provide a final emulsion having a 5% vitamin concentration (or other concentration as may be desired). The required additions of water can be made at this point without fear of altering the emulsion stability.

Chemical stability and emulsion stability are not impaired by exposure of the emulsion to a temperature of 120°C. for two hours, sufficient to sterilise by autoclaving.

Example 2

The procedure as described in Example 1 was repeated using in place of vitamin K_1, 50 grams of vitamin K_1 oxide (2-methyl-3-phytyl - 1,4 - naphthoquinone - 2,3 - oxide, a colorless oil somewhat more stable than K_1 but having the same physiological activity as K_1) and resulted in a stable emulsion which was not adversely affected by heating at a temperature of 120°C. for a two hour period.

Example 3

The procedure as described in Example 1 was repeated using in place of vitamin K_1, 50 grams of vitamin A; the stable emulsion thereby obtained was not adversely affected by heating at a temperature of 120°C. for two hours.

In addition to the foregoing, it has been found that stable emulsions of vitamin D and vitamin E can be prepared by employing the procedure as described in Example 1, merely substituting 50 grams of vitamin D and vitamin K, respectively for the vitamin K_1 referred to therein.

In addition to the advantage of not being adversely affected by extended periods of heating at 120°C., it should be noted that the vitamin emulsions have been stored for extended periods of time at room temperature and under refrigeration without showing any loss of stability. In addition, they have been shipped by train and lorry and subjected to the normal rough handling of commerce without affecting the physical stability of the emulsions.

What we claim is:—

1. A stable oily vitamin emulsion comprising an oily vitamin and lecithin dispersed in water, in which the dispersed particles are predominantly of a diameter in the range from 0.1 to 0.5 microns.

2. A stable oily vitamin emulsion according to Claim 1, comprising vitamins A, D, E, K_1 or vitamin K_1 oxide.

3. A stable oily vitamin emulsion according to Claims 1 or 2, comprising about 5% by weight of oily vitamin and about 1 to 2% by weight of lecithin dispersed in water.

4. A method for producing stable oily vitamin emulsions as claimed in Claim 1, which comprises admixing (1) a solution of

the oily vitamin in an aliphatic alcohol containing not more than four carbon atoms to the molecule, (2) a solution of lecithin in a volatile organic solvent, and (3) water, emulsifying the resulting mixture by agitation, and removing the organic solvents from the resulting emulsion.

5. A method according to Claim 4, in which the oily vitamin is vitamin A, D, E, K_1 or vitamin K_1 oxide.

6. A method according to Claims 4 or 5, in which the volume ratios of oily-vitamin: organic-solvents: water, are within the range of from 1 : 1 : 2 to about 1 : 5 : 12.

7. A method according to Claim 6, in which the oily vitamin, organic solvents, and water, are combined in the approximate volume ratio of one part oily vitamin to five parts of combined organic solvents to nine parts of water.

8. A method according to any one of Claims 4-7, in which the oily vitamin is initially introduced in isopropanol solution.

9. A method according to any one of Claims 4-8, in which the emulsion is produced by rapid stirring for a time sufficient to elevate its temperature to about 43 to 45°C.

10. A method according to any one of Claims 4-9, in which the organic solvents are removed by evaporation under reduced pressure.

11. A method according to any one of Claims 4-10, in which after removal of the organic solvents from the emulsion, further water is added to it.

12. A method according to any one of Claims 4-11, in which the aliphatic alcohol used to dissolve the oily vitamin is propanol or isopropanol.

13. A method for producing stable oily vitamin emulsions substantially as hereinbefore described with reference to any one of the foregoing examples.

14. Stable emulsions according to Claim 1, whenever prepared by a method particularly described and ascertained herein.

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